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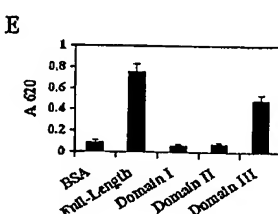
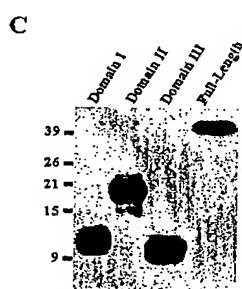
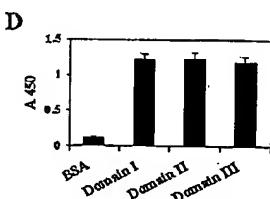
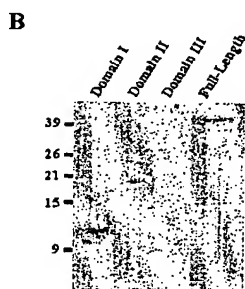
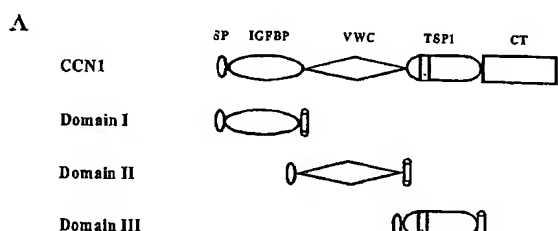
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[Continued on next page]

(54) Title: CCN1 COMPOSITIONS AND METHODS



(57) Abstract: The angiogenic inducer CCN1 (cysteine-rich 61, CYR61), a secreted matricellular protein of the CCN family, is a ligand of multiple integrins including  $\alpha_6\beta_1$ . Previous studies have shown that CCN1 interaction with integrin  $\alpha_6\beta_1$  mediates adhesion of fibroblasts, endothelial cells, and smooth muscle cells, as well as migration of smooth muscle cells. Recently, we have reported that CCN1-induced tubule formation of unactivated endothelial cells is also mediated through integrin  $\alpha_6\beta_1$ . In this study, we demonstrate that human skin fibroblasts adhere specifically to the T1 sequence (GQKCIQTTSWSQCSKS) within domain III of CCN1, and this process is blocked by anti- $\alpha_6$  and anti- $\beta_1$  monoclonal antibodies. Alanine substitution mutagenesis of the T1 sequence further defines the sequence TTWSQCSKS as the critical determinant for mediating  $\alpha_6\beta_1$ -dependent adhesion. Soluble T1 peptide specifically inhibits fibroblast adhesion to CCN1 in a dose-dependent manner. Furthermore, T1 also inhibits cell adhesion to other  $\alpha_6\beta_1$  ligands including CCN2 (CTGF), CCN3 (NOV), and laminin, but not to ligands of other integrins. In addition, T1 specifically inhibits  $\alpha_6\beta_1$ -dependent tubule formation of unactivated endothelial cells in a CCN1-containing collagen gel matrix. To confirm that T1 binds integrin  $\alpha_6\beta_1$  directly, we perform affinity chromatography and show that integrin  $\alpha_6\beta_1$  is isolated from an octylglucoside extract of fibroblasts on T1-coupled Affi-gel. Taken together, these findings define the T1 sequence in CCN1 as a novel binding motif for integrin  $\alpha_6\beta_1$ , and form the basis for the development of peptide mimetics to examine the functional role of  $\alpha_6\beta_1$  in angiogenesis.



SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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